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Infection With GB Virus C in Patients With Chronic Liver Disease

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Infection with putative non-A to E hepatitis virus, designated GB virus C (GBV-C), was surveyed in 286 patients with chronic liver disease in Japan. RNA of GBV-C was detected, by reverse-transcription polymerase chain reaction with nested primers from the 5′-noncoding region, in 19 patients (6.6%) at a frequency higher ($P <$ 0.001) than in three of 275 (1.1%) normal controls. It was detected in three of 83 (4%) patients with hepatitis B virus infection, 15 of 188 (8%) patients with hepatitis C virus infection, and one of 12 (8%) patients without evidence of ongoing infection with hepatitis B or C virus. GBV-C RNA was detected in nine of 186 (5%) patients with chronic hepatitis aged 51.2 ± 13.3 years, six of 64 (9%) with liver cirrhosis aged 62.9 ± 11.4 years, and four of 36 (11%) with hepatocellular carcinoma aged 62.0 ± 11.1 years. Nucleotide sequences of 100 base pairs in the helicase region of GBV-C isolates from the 19 patients varied up to 21%, while sequences of 33 deduced amino acids were conserved and differed only by up to 6%. These results indicate that infection with GBV-C in patients with non-B, non-C chronic liver disease would not be frequent, although the sensitivity of the detection method could be improved. Coinfection of GBV-C with hepatitis B or C virus, as well as the duration of infection, might accelerate the progression of chronic liver disease. ***J. Med. Virol. 51:175–181, 1997.***

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KEY WORDS: hepatitis virus; hepatitis B virus; hepatitis C virus; chronic hepatitis; blood transfusion

INTRODUCTION

The discovery of hepatitis C virus (HCV) is the major breakthrough in the history of non-A, non-B (NANB) hepatitis [Choo et al., 1989]. HCV is responsible for the majority of patients with blood-borne NANB hepatitis [Alter et al., 1989; Kuo et al., 1989]. As serological tests for HCV infection became routine in clinical practice and for screening blood units, however, it has become increasingly evident that there may be an additional viral agent(s) which causes community-acquired or posttransfusion NANB hepatitis [Alter and Bradley, 1995]. This category of hepatitis is referred to as non-A to E on the premise that it is also not related to hepatitis D or E virus.

Deinhardt et al. [1967] reported that the sera from a surgeon (GB) with hepatitis and a retrospective diagnosis of acute non-A to E hepatitis induced hepatocellular injury in primates. The GB serum has been propagated in tamarins, and viral agents were cloned and characterized as GB viruses A and B [Simons et al., 1995b]. Recently, Simons et al. [1995a] have detected and partially sequenced a virus, which has sequence similarity to GB viruses A and B, and named it GB virus C (GBV-C). They detected GBV-C in sera from eight individuals living in various districts of the world, three of whom had acute or chronic non-A to E hepatitis. Further, Leary et al. [1996] established the genomic sequence and organization of GBV-C. Recently, Linnen et al. [1996] reported two isolates of hepatitis G virus. GBV-C and hepatitis G virus share 86% of nucleotide and 96–97% of amino acid sequences; therefore, they would be the same virus of possibly distinct genotypes.

The genomic organization of GBV-C is similar to that of the *Flaviviridae* family [Simons et al., 1995a]. Its sequence resembles that of HCV, in particular, al-

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though with too wide a divergence to be classified as genotypes of HCV. An early disclosure of a partial sequence of the GBV-C genome by Simons et al. [1995a] enabled the detection of this virus by means of reverse-transcription polymerase chain reaction (RT-PCR) with primers deduced from the helicase region [Simons et al., 1995a; Yoshiba et al., 1995].

Since GBV-C RNA has been identified in some patients with fulminant hepatitis or chronic hepatitis without any evidence for known hepatitis virus infection [Simons et al., 1995a; Yoshiba et al., 1995], it would qualify as a non-ABC hepatitis virus. It is yet to be seen, however, how GBV-C accounts for chronic hepatitis in patients who are without serological markers of infection with known hepatitis viruses. For the purpose of evaluating the contribution of GBV-C to chronic hepatitis and its sequelae, patients who received care in a city hospital in Japan were surveyed for the viral RNA. The results were compared with the serological markers of hepatitis B virus (HBV) and HCV infections so as to evaluate the contribution of GBV-C to chronic non-ABC hepatitis and the effect of its coinfection on patients infected with defined hepatitis viruses.

MATERIALS AND METHODS

Patients

During 7 months, from January to July 1995, 286 consecutive patients with chronic liver disease of a defined or suspected viral etiology received care at the Department of Internal Medicine, Iwaki Kyoritsu General Hospital. They were aged 55.2 ± 13.8 years and included 165 males and 121 females. None had evidence of metabolic, autoimmune, or toxic liver disease, nor were they positive for antibody to human immunodeficiency virus type 1. Chronic hepatitis was diagnosed in 186 patients, liver cirrhosis in 64, and hepatocellular carcinoma in the remaining 36. The diagnosis of liver disease was based on clinical, chemical, and ultrasonographic findings, which was confirmed by liver biopsy in the majority of patients. They were interviewed for known risk factors for blood-borne viral infections in the past, such as a history of transfusion and illicit intravenous drugs; and their sera were tested for serological markers of hepatitis viruses and RNA of GBV-C. The study was approved by the Ethics Committee of the institution, and informed consent was obtained from every patient.

Detection of GBV-C RNA

Total nucleic acids were extracted from serum (100 μl) with guanidinium isothiocyanate and phenol (ISOGEN-LS; Nippon Gene Co., Tokyo, Japan) and dissolved in 5.3 μl of distilled water treated with diethylpyrocarbonate. They were heated at 70°C for 1 min, chilled quickly on ice, and subjected to cDNA synthesis with reverse transcriptase (Superscript II; GIBCO-BRL, Gaithersburg, MD) and antisense primer #G75 with a sequence of 5′-CCTATTGGTCAAGAGAGACAT-3′. Reverse-transcribed cDNAs were heated at 95°C for 15 min, and a half-portion was subjected to first-round PCR with sense primers #G58, sequenced 5′-CAGGGTTGGTAGGTCGTAAATCC-3′, and #G75 for 35 cycles (94°C, 30 sec; 55°C, 30 sec; 72°C, 60 sec [8 min in the last cycle]). One-tenth amplification products of the first round of PCR, with a size of 242 base pairs, was subjected to the second round of PCR with nested primers, sense #G134, sequenced 5′-GGTCAYCYTG-GTAGCCACTATAGG-3′ (Y = C or T), and antisense #G131, sequenced 5′-AAGAGAGACATTGWAGGG-CGACGT-3′ (W = A or T), for 25 cycles (94°C, 30 sec; 55°C, 30 sec; 72°C, 60 sec [8 min in that last cycle]) to amplify a fragment of 208 base pairs. All primers were deduced from conserved areas in the 5′-noncoding regions of GBV-C or hepatitis G virus (GenBank/EMBL/DDBJ accession numbers: U36380, U44402, U45966, D90600, and D90601). PCR was carried out in accordance with the guidelines of Kwok and Higuchi [1989] to avoid contamination, with one positive and two negative controls inserted every 20 samples.

Sequence Analysis

A nucleotide sequence spanning 100 base pairs in the helicase region was determined for sera containing GBV-C RNA. cDNA of GBV-C amplified by PCR with heminested primers #G8 (sense) and #G11 (antisense) deduced from the helicase region [Yoshiba et al., 1995; Masuko et al., 1996] was treated with T4 DNA polymerase (TaKaRa Biochemicals, Kyoto, Japan) and T4 polynucleotide kinase (New England Biolabs, Beverly, MA), cloned into M13 phage vectors that had been cleaved with *Hin*cII (TaKaRa Biochemicals) and dephosphorylated. The sequence of GBV-C cDNA was then determined by the dideoxy-chain termination method with an ALF AutoRead DNA sequencing kit (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Serological Tests

Hepatitis B surface antigen (HBsAg) was determined by passive hemagglutination using commercial kits (MyCell, Institute of Immunology Co., Tokyo, Japan). Antibody to hepatitis B core (anti-HBc) was determined by hemagglutination inhibition after the method described previously [Iizuka et al., 1992]. HBV DNA was determined in nucleic acids extracted from 100 μl of serum by PCR with nested primers deduced from the S gene by the method described elsewhere [Iizuka et al., 1992]. Antibody to HCV (anti-HCV) was determined by enzyme immunoassay of the second generation (EIA-II; Ortho Diagnostic Systems, Tokyo, Japan), with A_{492} readings >0.63 considered reactive. Serum samples positive by EIA-II were tested for HCV RNA by RT-PCR with primers deduced from conserved areas in the 5′-noncoding region of HCV [Okamoto et al., 1994].

TABLE I. RNA of GB Virus C in Sera From Patients With Chronic Liver Disease Infected With HBV, HCV, Both, or Neither

Etiology	N	Age (years)	Male	Trans-fusion	GBV-C[a] RNA
HBV	83	45.8 ± 12.5	57 (69%)	9 (11%)	3 (4%)
HCV	188	58.9 ± 12.1	98 (52%)	84 (45%)	15 (8%)
HBV and HCV	3	64.0 ± 7.9	3 (100%)	0	0
Non-B, non-C	12	58.8 ± 16.9	7 (58%)	0	1 (8%)
Total	286	55.2 ± 13.8	165 (57.7%)	93 (32.5%)	19 (6.6%)

[a]RNA of GB virus C was determined by reverse-transcription polymerase chain reaction with nested primers deduced from the 5′-noncoding region (see "Materials and Methods").

Statistical Analyses

The frequency between groups was compared using the χ^2 test and Fisher's exact test. Group means were compared by Student's *t* test.

RESULTS

Etiology of Chronic Liver Disease in the 286 Patients

The 286 patients were classified by the etiology of liver disease. They were considered to have ongoing HBV infection if HBsAg was present in their sera. If HBsAg was not detected in serum, the diagnosis of ongoing HBV infection was indicated by high-titered anti-HBc accompanied by HBV DNA. Ongoing HCV infection was defined by the detection of HCV RNA in serum along with anti-HCV in high titers. The absence of current HBV infection was judged in the patients who were negative for HBsAg or anti-HBc or when they had anti-HBc (usually in low titers) by the failure to detect HBV DNA in serum. The absence of concurrent HCV infection was deduced by negative HCV RNA in serum. Thus, 83 of the 286 (29%) patients were positive for HBV infection, 188 (66%) were positive for HCV infection, and three (1%) had both HBV and HCV infections. The remaining 12 (4%) patients had no evidence for ongoing infection with HBV or HCV, and they were estimated to have non-B, non-C liver disease.

GBV-C Infection in Patients With Chronic Liver Disease

Table I compares demographic features, previous transfusions, and the detection of GBV-C RNA in sera from patients with chronic liver disease of four etiologies, i.e., HBV, HCV, both (HBV and HCV), and neither (non-B, non-C). None of them reported use of illicit intravenous drugs in the past. GBV-C RNA was detected in three patients (4%) with HBV infection, 15 (8%) with HCV infection, and one (8%) without evidence for ongoing HBV or HCV infection (non-B, non-C); it was not detected in any of three patients with both HBV and HCV infections. Overall, GBV-C RNA was detected in 19 of the 286 (6.6%) patients with chronic liver disease, more frequently than in three of 275 (1.1%) apparently healthy blood donors without elevated transaminase levels ($P < 0.001$). Patients with HCV infection were older ($P < 0.001$) and had a history of transfusion more frequently ($P < 0.001$) than those with HBV infection.

Table II compares the detection of GBV-C RNA in sera from patients with chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. GBV-C RNA was detected somewhat less frequently in patients with chronic hepatitis (nine of 186 or 5%) than in patients with liver cirrhosis or hepatocellular carcinoma (10 of 100 or 10%). However, patients with chronic hepatitis were significantly younger than those with liver cirrhosis or hepatocellular carcinoma (51.2 ± 13.3 vs. 62.6 ± 11.2 years, $P < 0.001$).

Features of the 19 patients with GBV-C RNA in serum and the 267 patients without it are compared in Table III. There were no appreciable differences in demographics, liver function, history of transfusion, etiology, or severity of liver disease between the two groups of patients.

Nucleotide and Deduced Amino Acid Sequences of GBV-C

Sequences of 100 base pairs in the helicase region of GBV-C isolates from the 19 patients are shown in Figure 1 in comparison with those from eight inhabitants of Africa, Canada, and the United States, including three patients with acute or chronic non-ABC hepatitis originally reported by Simons et al. [1995a], as well as three Japanese patients with fulminant non-ABC hepatitis [Yoshiba et al., 1995].

Similarities of 79–98% (mean 86.7 ± 3.2%) were observed among GBV-C isolates from the 19 patients, 77–99% (83.4 ± 4.9%) among eight isolates reported by Simons et al. [1995a], and 80–86% (83.3 ± 3.1%) among isolates from three Japanese patients with fulminant hepatitis [Yoshiba et al., 1995]. Sequences of GBV-C isolates from the 19 patients were similar to those of the three Japanese isolates in 85.1 ± 2.6% (range 78–89%), more closely ($P < 0.001$) than to those reported by Simons et al. [1995a] in 81.6 ± 3.1% (75–90%).

Sequences of the 33 deduced amino acids in the helicase region are compared in Figure 2. Sequences of GBV-C isolates from 14 patients (cases 1–14) were identical to the three Japanese isolates [Yoshiba et al., 1995] and a single isolate (GBV-C.2) reported by Simons et al. [1995a]. The sequence from one patient (case 17) was identical to four isolates of Simons et al. [1995a] (GBV-C, GBV-C.1, GBV-C.3, and GBV-C.5). Sequences from the remaining three patients were distinct from those previously reported; they differed from

TABLE II. RNA of GB Virus C in Sera From Patients With Chronic Hepatitis, Liver Cirrhosis, or Hepatocellular Carcinoma

Disease	N	Age (years)	Male	Transfusion	GBV-C RNA
Chronic hepatitis	186	51.2 ± 13.3	104 (56%)	56 (30%)	9 (5%)
Liver cirrhosis	64	62.9 ± 11.4	35 (55%)	28 (44%)	6 (9%)
Hepatocellular carcinoma	36	62.0 ± 11.1	26 (72%)	9 (25%)	4 (11%)
Total	286	55.2 ± 13.8	165 (57.7%)	93 (32.5%)	19 (6.6%)

TABLE III. Demographic, Clinical, and Viral Features of Patients With Chronic Liver Disease Infected and Not Infected With GB Virus C

Features	GBV-C RNA Positive (n = 19)	GBV-C RNA Negative (n = 267)	Differences
Age (years)	57.2 ± 15.7	55.0 ± 13.6	NS[a]
Male	11 (58%)	154 (58%)	NS
Disease			
Hepatitis	9 (47%)	175 (66%)	NS
Cirrhosis	6 (32%)	58 (22%)	NS
Carcinoma	4 (21%)	32 (12%)	NS
Liver function[b]			
ALT (IU/L)	31.8 ± 24.1	38.1 ± 43.3	NS
AST (IU/L)	38.9 ± 18.1	46.6 ± 34.3	NS
ZTT (Kunkel U)	17.0 ± 4.7	17.0 ± 7.7	NS
Transfusion	7 (37%)	86 (32%)	NS
Etiology			
HBV	3 (16%)	80 (30%)	NS
HCV	15 (79%)	173 (65%)	NS
B and C	0	3 (1%)	NS
Non-B, non-C	1 (5%)	11 (4%)	NS

[a]Not significant.
[b]ALT, alanine aminotransferase (normal 3–20 IU/l); AST, aspartate aminotransferase (8–25 IU/l); ZTT, zinc turbidity tests (3–13 Kunkel units).

those of the other 16 patients studied or the three Japanese patients with fulminant hepatitis by one amino acid and from those of eight isolates reported by Simons et al. [1995a] by up to three amino acids.

DISCUSSION

Although HCV is the major etiologic agent for chronic NANB hepatitis, there are patients with this disease who are without serological or molecular biological markers of HCV infection. Chemello et al. [1993] could not detect anti-HCV by the second-generation EIA in 26 (9.3%) biopsy-proven chronic NANB hepatitis. Kodali et al. [1994] could not determine a definite etiology in 28 of 567 (4.9%) patients with chronic liver disease and categorized them as cryptogenic. In the present series of 286 patients with chronic liver disease who were without evidence of a toxic, metabolic, or autoimmune cause, 12 (4.2%) did not have serological or molecular biological evidence of HBV or HCV infection.

When sera from the 286 patients were tested for GBV-C RNA by RT-PCR with nested primers from the 5′-noncoding region, it was detected in 19 (6.6%), significantly more frequently ($P < 0.001$) than in three of 275 (1.1%) blood donors. However, 18 of the 19 patients with GBV-C RNA were infected with HBV or HCV, leaving only one (8%) among 12 patients with chronic non-ABC liver disease who was infected with GBV-C. Our results stand at variance with those of Fiordalisi et al. [1996], who detected GBV-C RNA by RT-PCR with helicase region primers in seven of 18 (39%) patients with chronic non-A to E hepatitis in Italy, much more frequently than in only one of 100 (1%) blood donors there.

Taken at face value, the contribution of GBV-C to non-ABC hepatitis would not be high, although there may be some regional differences. We used primers deduced from the 5′-noncoding region in PCR for the detection of GBV-C RNA. This region is expected to be highly conserved in GBV-C; primers deduced from the 5′-noncoding region are sensitive in the detection of HCV RNA [Okamoto et al., 1990]. Within the sequence of 100 base pairs in the helicase region, divergences of 1–23% is observed among GBV-C isolates from different countries and 16–20% even among those from Japan. In the patients studied herein, GBV-C sequences differed by up to 21%, although they were more close to the isolates from Japan [Yoshiba et al., 1995] than to those from Africa, Canada, and the United States [Simons et al., 1995a]. Despite such marked divergence in the nucleotide sequence, the sequence of 33 amino acids deduced from 100 nucleotides in the helicase region was very well conserved. Sequences of GBV-C isolates from 15 of the 19 patients were the same and differed only by one or two (3–6%) amino acids from those of carriers from different countries.

There were no appreciable differences in liver function between the 19 patients with GBV-C RNA in serum and the 267 without. Since the majority (95%) of patients with GBV-C RNA were coinfected with HBV or HCV, the effect of GBV-C per se could not be evaluated in them. Non-A to E hepatitis tends to run a mild course regardless of its development, either sporadically or after transfusions [Alter and Bradley, 1995]. This view would be in line with no elevation in serum transaminases in the three individuals with GBV-C identified among 275 Japanese blood donors. However, non-A to E hepatitis viruses are incriminated in most cases of fulminant hepatitis nowadays [Fagan and Harrison, 1994; Mutimer et al., 1995; Wright et al., 1991]. In actuality, GBV-C RNA has been detected in some patients with fulminant hepatitis of unknown eti-

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                     *         *         *         *         *         *         *         *         *         *
GBV-C       CGAGCGTATGAGGACTGGTCGCCACCTTGTATTCTGCCATTCCAAGGCGGAGTGCGAGAGATTGGCCGGCCAGTTCTCCGCGCGGGGGGTTAATGCCATC
GBV-C.1     ------------------------------------------------------------------T---------------------------------
GBV-C.2     ------G---C----C--G--------C--G-----------A---------------C-G-----T-----------TT----------G--------T
GBV-C.3     G---A-G---C----C--CA-G-----C--------------A---------------C-G--T--T-----------A--C--------A--------T
GBV-C.4     G-----G--------C---A-G-----G-----------C--A-----------T-----GC----------A------T-A--------------TG-T
GBV-C.5     ---A--G---C----C--AA-G-----C--G-----------A---------------C-GC-C--T--T-----T--T---A-------A--C-----T
GBV-C.6     G-----G---CA---C---A-A--T-----G---C----C--G---------------C-GC-T---------------T-TA-------C--C-----T
GBV-C.7     ---A--G---C-A--C--AG-G-----C--G-----T---------------------C-GC-T--T-----------T---A-------G--------T

FH #1       T-----G---C-A--C--A--G-----C--G-----------------T---------C-CC-C-----T--------TT-TA----A--C-----T---
FH #2       G-----G---C-A--C--CA-G--------G--T--T-----A-----A---------C-CC----T--------T---T-TA-------C--C------
FH #3       G-----G---C-A--C--CA-G---T-G--------------A---------------C-C-----------A-----T-----------A--C-----A

Case  1     G-----G---C----C--CA-G--T--C--G--------C--A---------------C-TC----G--------T--GT-A--T-----C--------T
Case  2     G-----G---C----C--CA-G--T--C--G-----T--C--A---------------C-TC----G--------T--GT-A--T--------------T
Case  3     G-----G---C-A--A--CA-G-----C--------------A---------------C-CC----T--G--A------T-C-----T--C---------
Case  4     G--A--G---C----C--CA-G-----C--G--T-----C------------------C-CC----T-----------GT-CA----A--------T--T
Case  5     G-----G---C-------CA-G--T--C-----------C--A--------------AC-TC----------A------T-T--------C--------T
Case  6     G-----G---C-T--C--CA-G--T--C--------------T---------------C-CC----G-----A------T-TA----T--C-----T---
Case  7     G---A-G---C----C--GA-G--T--C--G-----------T---------------C-CC----G-----------TT-C-----------------T
Case  8     G-----G---C----C--GA-G--T--C-----------------------------AC-CC----G-----A------T-T--------C-----T--T
Case  9     G---A-G---C-------GA-G-----C--G--T--------T---------------C-CC----A-----A-----TT-C--------C--C-----A
Case 10     G-----G---C-A--C--C--G--T--C--G--------C--A--------------AC-CC-------------T---T-TA----T--A-----T--T
Case 11     G-----G---C----C--A--A--T--C--G--------C--A---------------C-T--------T-----T---T-TA-------A--C-----T
Case 12     G-----G---C----C---A-G--T--C--G-----T-----A-----------T---C-CC----G-----A------T-TA-------A--------T
Case 13     G-----G---C-A--C---A-G-----C-----------C--A---------------C-CC-------------T---T-CA-------C-----T--T
Case 14     G-----G---C-C------A-G--T--C-----------C--A---------------C-TC----A-----A------T-C-----------------T
Case 15     G-----G---C--------A-G--T--C--------------T-----------T---C-CC----A-----A------T-C--------C--------T
Case 16     G-----G---C-T--C--CA-G-----CA----T--------T---------------C-C-----G-----A------T-TA-------G--------A
Case 17     ------G---C----C---A-G--T--C--G--------C------------------C-G--------T-----T-----TA----T--C--C-----T
Case 18     G-----G---C-A--C---A-G-----C--------------G---------------C-CC-----------------C-CA-------A---------
Case 19     G-----G---C-A--C--C--G---T-G--G---------------------------C-TC----G--------T--GA-CA-------C--------A
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Fig. 1. Nucleotide sequence of 100 base pairs in the helicase region of GB virus C (GBV-C). Sequences of GBV-C isolates from Africa (GBV-C, GBV-C.1–4), Canada (GBV-C.5), and the United States (GBV-C.6 and 7), as reported by Simons et al. [1995a], are indicated above and those from three Japanese patients with fulminant hepatitis of unknown etiology (FH#1–3), as reported by Yoshiba et al. [1995], in the middle. The consensus sequences of three GBV-C clones from each of the 19 patients with chronic liver disease are indicated below.

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                     *         *         *
GBV-C       ERMRTGRHLVFCHSKAECERLAGQFSARGVNAI
GBV-C.1     ---------------------------------
GBV-C.2     --------------------------S------
GBV-C.3     ---------------------------------
GBV-C.4     --------------------------S-----V
GBV-C.5     ---------------------------------
GBV-C.6     ---Q-------R--------------S------
GBV-C.7     ------G--------------------------

FH #1       --------------------------S------
FH #2       --------------------------S------
FH #3       --------------------------S------

Case  1     --------------------------S------
Case  2     --------------------------S------
Case  3     --------------------------S------
Case  4     --------------------------S------
Case  5     --------------------------S------
Case  6     --------------------------S------
Case  7     --------------------------S------
Case  8     --------------------------S------
Case  9     --------------------------S------
Case 10     --------------------------S------
Case 11     --------------------------S------
Case 12     --------------------------S------
Case 13     --------------------------S------
Case 14     --------------------------S------
Case 15     --------------------------S------
Case 16     ---------I----------------S------
Case 17     ---------------------------------
Case 18     --------------------------P------
Case 19     --------------------------T------
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Fig. 2. Amino acid sequence of a part of the helicase region of GB virus C (GBV-C). Sequences of 33 amino acids of GBV-C isolates from the 19 patients are compared with those reported by Simons et al. [1995a] and those from Japanese patients with fulminant hepatitis of unknown etiology reported by Yoshiba et al. [1995]. Single letter codes for amino acids are: A, alanine; C, cysteine; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine, T, threonine; V, valine.

ology [Yoshiba et al., 1995]. Hence, most GBV-C strains may induce mild or subclinical liver injuries, while rare GBV-C variants might have mutations for active replication and high disease-inducing activity, or there could be additional factor(s) for the development of severe hepatic injuries in persons infected with GBV-C.

The past 20 years have witnessed the discovery of five hepatitis viruses, from A to E, and more may yet be added to the list. The time has come when many hepatitis viruses have to be considered in dealing with the disease in a single patient. Due to the blood-borne nature of HBV, HCV, and GBV-C, they may coinfect in certain populations at increased risk. Infection with multiple hepatitis viruses would not be rare [Purcell, 1994], and more than 10% of HBV carriers in the world are estimated to be coinfected with HCV [Liaw, 1995]. This view is corroborated by the detection of GBV-C RNA in 15 of 188 (8%) patients with HCV infection and three of 83 (4%) patients with HBV infection in the present study.

Hepatitis viruses may interact with each other, which can be synergetic, antagonistic, or additive, to modify the clinical picture. Infection with HCV is reported to suppress the replication of HBV and vice versa to a lesser extent [Koike et al., 1995; Liaw et al., 1994; Pontisso et al., 1993]. Despite this viral interference, coinfection with HBV and HCV aggravates liver disease [Colombari et al., 1993; Crespo et al., 1994; Fong et al., 1991], increases the risk for developing hepatocellular carcinoma [Benvegnu et al., 1994; Kaklamani et al., 1991] as well as fulminant hepatitis [Chu et al., 1994; Wu et al., 1994], and leads to a poor response to interferon [Weltman et al., 1995]. GBV-C RNA was detected more frequently in patients with liver cirrhosis or hepatocellular carcinoma than in

those with chronic hepatitis in the present study. This could reflect a possible role of GBV-C in aggravating liver disease in cooperation with the other hepatitis viruses. The studied patients with chronic hepatitis were younger than those with liver cirrhosis or hepatocellular carcinoma, however, leaving the duration of disease as another contributing factor for the progression of liver disease.

In Western countries, HCV infection is detected rarely, if ever, in patients with NANB fulminant hepatitis [Fagan and Harrison, 1994; Mutimer et al., 1995; Wright et al., 1991]. This is in sharp contrast to the detection of anti-HCV and HCV RNA in a significant proportion of the patients with this disease among the Japanese [Yanagi et al., 1991; Yoshiba et al., 1994] and Americans of Hispanic ancestry [Villamil et al., 1995]. It remains to be seen whether or not GBV-C aggravates liver disease in individuals who are infected with HCV toward the development of fulminant hepatitis. Despite the initial expectation that HCV would be responsible for aplastic anemia in patients with hepatitis, the virus is not implicated in later studies [Hibbs et al., 1992; Pol et al., 1990]. It would be worthwhile to see if GBV-C is accountable for aplastic anemia of a non-ABC etiology, as it has been reported in some patients recently [Byrnes et al., 1996; Zaidi et al., 1996].

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REFERENCES

Alter HJ, Bradley DW (1995): Non-A, non-B hepatitis unrelated to the hepatitis C virus (non-ABC). Seminars in Liver Disease 15:110–120.

Alter HJ, Purcell RH, Shih JW, Melpolder JC, Houghton M, Choo QL, Kuo G (1989): Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. New England Journal of Medicine 321: 1494–1500.

Benvegnu L, Fattovich G, Noventa F, Tremolada F, Chemello L, Cecchetto A, Alberti A (1994): Concurrent hepatitis B and C virus infection and risk of hepatocellular carcinoma in cirrhosis. A prospective study. Cancer 74:2442–2448.

Byrnes JJ, Banks AT, Piatack MJ, Kim JP (1996): Hepatitis G-associated aplastic anaemia [Letter]. Lancet 348:472.

Chemello L, Cavalletto D, Pontisso P, Bortolotti F, Donada C, Donadon V, Frezza M, Cesarin P, Alberti A (1993): Patterns of antibodies to hepatitis C virus in patients with chronic non-A, non-B hepatitis and their relationship to viral replication and liver disease. Hepatology 17:179–182.

Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M (1989): Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244:359–362.

Chu CM, Sheen IS, Liaw YF (1994): The role of hepatitis C virus in fulminant viral hepatitis in an area with endemic hepatitis A and B. Gastroenterology 107:189–195.

Colombari R, Dhillon AP, Piazzola E, Tomezzoli AA, Angelini GP, Capra F, Tomba A, Scheuer PJ (1993): Chronic hepatitis in multiple virus infection: Histopathological evaluation. Histopathology 22:319–325.

Crespo J, Lozano JL, de la Cruz F, Rodrigo L, Rodriguez M, San MG, Artinano, E, Pons RF (1994): Prevalence and significance of hepatitis C viremia in chronic active hepatitis B. American Journal of Gastroenterology 89:1147–1151.

Deinhardt F, Holmes AW, Capps RB, Popper H (1967): Studies on the transmission of human viral hepatitis to marmoset monkeys. I. Transmission of disease, serial passages, and description of liver lesions. Journal of Experimental Medicine 125:673–688.

Fagan EA, Harrison TJ (1994): Exclusion in liver by polymerase chain reaction of hepatitis B and C viruses in acute liver failure attributed to sporadic non-A, non-B hepatitis. Journal of Hepatology 21:587–591.

Fiordalisi G, Zanella I, Mantero G, Bettinardi A, Stellini R, Paraninfo G, Cadeo G, Primi D (1996): High prevalence of GB virus C infection in a group of Italian patients with hepatitis of unknown etiology. Journal of Infectious Diseases 176:181–183.

Fong TL, Di Bisceglie AM, Waggoner JG, Banks SM, Hoofnagle JH (1991): The significance of antibody to hepatitis C virus in patients with chronic hepatitis B. Hepatology 14:64–67.

Hibbs JR, Frickhofen N, Rosenfeld SJ, Feinstone SM, Kojima S, Bacigalupo A, Locasciulli A, Tzakis AG, Alter HJ, Young NS (1992): Aplastic anemia and viral hepatitis. Non-A, non-B, non-C? Journal of the American Medical Association 267:2051–2054.

Iizuka H, Ohmura K, Ishijima A, Satoh K, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M (1992): Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. Vox Sanguinis 63:107–111.

Kaklamani E, Trichopoulos D, Tzonou A, Zavitsanos X, Koumantaki Y, Hatzakis A, Hsieh CC, Hatziyannis S (1991): Hepatitis B and C viruses and their interaction in the origin of hepatocellular carcinoma. Journal of the American Medical Association 265:1974–1976.

Kodali VP, Gordon SC, Silverman AL, McCray DG (1994): Cryptogenic liver disease in the United States: Further evidence for non-A, non-B, and non-C hepatitis. American Journal of Gastroenterology 89:1836–1839.

Koike K, Yasuda K, Yotsuyanagi H, Moriya K, Hino K, Kurokawa K, Iino S (1995): Dominant replication of either virus in dual infection with hepatitis viruses B and C. Journal of Medical Virology 45: 236–239.

Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradley DW, Houghton M (1989): An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 244:362–364.

Kwok S, Higuchi R (1989): Avoiding false positives with PCR. Nature 339:237–238.

Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schalauder GG, Dawson GJ, Desai SM, Mushahwar IK (1996): Sequence and genomic organization of GBV-C: A novel member of the Flaviviridae associated with human non-A-E hepatitis. Journal of Medical Virology 48:60–67.

Liaw YF (1995): Role of hepatitis C virus in dual and triple hepatitis virus infection. Hepatology 22:1101–1108.

Liaw YF, Tsai SL, Chang JJ, Sheen IS, Chien RN, Lin DY, Chu CM (1994): Displacement of hepatitis B virus by hepatitis C virus as the cause of continuing chronic hepatitis. Gastroenterology 106: 1048–1053.

Linnen J, Wages J, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JWK, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley D, Margolis H, Kim JP (1996): Molecular cloning and disease association of hepatitis G virus: A transfusion-transmissible agent. Science 271:505–508.

Masuko K, Mitsui T, Iwano K, Yamazaki C, Okuda K, Meguro T, Murayama N, Inoue T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M (1996): Infection with hepatitis GB virus C in patients on maintenance hemodialysis. New England Journal of Medicine 334:1485–1490.

Mutimer D, Shaw J, Neuberger J, Skidmore S, Martin B, Hubscher S, McMaster P, Elias E (1995): Failure to incriminate hepatitis B, hepatitis C, and hepatitis E viruses in the aetiology of fulminant non-A non-B hepatitis. Gut 36:433–436.

Okamoto H, Okada S, Sugiyama Y, Tanaka T, Sugai Y, Akahane Y, Machida A, Mishiro S, Yoshizawa H, Miyakawa Y, Mayumi M (1990): Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5′-noncoding region. Japanese Journal of Experimental Medicine 60:215–222.

Okamoto H, Mishiro S, Tokita H, Tsuda F, Miyakawa Y, Mayumi M (1994): Superinfection of chimpanzees carrying hepatitis C virus of genotype II/1b with that of genotype III/2a or I/1a. Hepatology 20:1131–1136.

Pol S, Driss F, Devergie A, Brechot C, Berthelot P, Gluckman E (1990): Is hepatitis C virus involved in hepatitis-associated aplastic anemia? Annals of Internal Medicine 113:435–437.

Pontisso P, Ruvoletto MG, Fattovich G, Chemello L, Gallorini A, Ruol A, Alberti A (1993): Clinical and virological profiles in patients with multiple hepatitis virus infections. Gastroenterology 105: 1529–1533.

Purcell RH (1994): Hepatitis viruses: Changing patterns of human disease. Proceedings of the National Academy of Sciences USA 91:2401–2406.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK (1995a): Isolation of novel virus-like sequences associated with human hepatitis. Nature Medicine 1:564–569.

Simons JN, Pilot-Matias TJ, Leary TP, Dawson GJ, Desai SM, Schlauder GG, Muerhoff AS, Erker JC, Buijk SL, Chalmers ML, Van Sant C, Mushahwar IK (1995b): Identification of two flavivirus-like genomes in the GB hepatitis agent. Proceedings of the National Academy of Sciences USA 92:3401–3405.

Villamil FG, Hu KQ, Yu CH, Lee CH, Rojter SE, Podesta LG, Makowka L, Geller SA, Vierling JM (1995): Detection of hepatitis C virus with RNA polymerase chain reaction in fulminant hepatic failure. Hepatology 22:1379–1386.

Weltman MD, Brotodihardjo A, Crewe EB, Farrell GC, Bilous M, Grierson JM, Liddle C (1995): Coinfection with hepatitis B and C or B, C and delta viruses results in severe chronic liver disease and responds poorly to interferon-alpha treatment. Journal of Viral Hepatitis 2:39–45.

Wright TL, Hsu H, Donegan E, Feinstone S, Greenberg H, Read A, Ascher NL, Roberts JP, Lake JR (1991): Hepatitis C virus not found in fulminant non-A, non-B hepatitis. Annals of Internal Medicine 115:111–112.

Wu JC, Chen CL, Hou MC, Chen TZ, Lee SD, Lo KJ (1994): Multiple viral infection as the most common cause of fulminant and subfulminant viral hepatitis in an area endemic for hepatitis B: Application and limitations of the polymerase chain reaction. Hepatology 19:836–840.

Yanagi M, Kaneko S, Unoura M, Murakami S, Kobayashi K, Sugihara J, Ohnishi H, Muto Y (1991): Hepatitis C virus in fulminant hepatic failure [Letter]. New England Journal of Medicine 324:1895–1896.

Yoshiba M, Dehara K, Inoue K, Okamoto H, Mayumi M (1994): Contribution of hepatitis C virus to non-A, non-B fulminant hepatitis in Japan. Hepatology 19:829–835.

Yoshiba M, Okamoto H, Mishiro S (1995): Detection of the GBV-V hepatitis virus genome in serum from patients with fulminant hepatitis of unknown aetiology. Lancet 346:1131–1132.

Zaidi Y, Chapman CS, Myint S (1996): Aplastic anaemia after HGV infection [Letter]. Lancet 348:471–472.